Thermal inactivation and sublethal injury kinetics of *Salmonella enterica* and *Listeria monocytogenes* in broth versus agar surface

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Abstract

The objective of the present study was to compare the thermal inactivation and sublethal injury kinetics of *Salmonella enterica* and *Listeria monocytogenes* in broth (suspended cells) and on solid surface (agar-seeded cells). A 3-strain cocktail of *S. enterica* or *L. monocytogenes* inoculated in broth or on agar was subjected to heating in a water bath at various set temperatures (55.0, 57.5 and 60.0°C for *S. enterica* and 60.0, 62.5 and 65°C for *L. monocytogenes*). The occurrence of sublethally injured cells was determined by comparing enumerations on nonselective (TSAYE) and selective (XLD or ALOA) media. Results showed that the inactivation curves obtained from selective media were log-linear, and significant shoulders ($p < 0.05$) were observed on some of the inactivation curves from TSAYE media. The *D*-values derived from the total population were higher than those from the uninjured cells. Generally, cells on agar surface exhibited higher heat resistance than those in broth. For *S. enterica*, cell injury increased with the exposure time, no difference was observed when treated at temperatures from 55.0 to 60.0°C, while for *L. monocytogenes*, cell injury increased significantly with heating time and treatment temperature (from 60.0 to 65°C). Moreover, the degree of sublethal injury affected by thermal treatment in broth or on agar surface depended upon the target microorganism. Higher proportions of injured *S. enterica* cells were observed for treatment in broth than on agar surface, while the opposite was found for *L. monocytogenes*. The provided information may be used to assess the efficacy of thermal treatment processes on surfaces for inactivation of *S. enterica* and *L. monocytogenes*, and it provides insight into the sublethally injured survival state of *S. enterica* and *L. monocytogenes* treated in liquid or on solid food.

Keywords: *D*-value; heat resistance; model agar system; surface pasteurization; meta-analysis

1. Introduction

The potential for food to harbour foodborne pathogens such as *Salmonella enterica* and *Listeria monocytogenes* is commonly reported (EFSA and ECDC, 2015; Rose et al., 2002). It is believed that consuming contaminated raw or undercooked food is one of the important vehicles of food borne infection and outbreaks (EFSA and ECDC, 2015; Harris et al., 2003). Therefore, adequate intervention processes are needed to eliminate foodborne pathogens and to ensure products are safe for consumption.

Currently, thermal processing remains the principal and traditional method of microbial inactivation for consumers and food industry (Álvarez-Ordóñez et al., 2008; Rawson et al., 2011). The use of heat to achieve a specific lethality is one of the important critical control points in reducing the risks associated with food borne pathogens. Nevertheless, the use of excessive heat to inactivate pathogens adversely affects important constituents of foods (such as vitamins and other micronutrients) as well as its sensory attributes (Bermúdez-Aguirre and Corradini, 2012; Devlieghere et al., 2004; Walsh et al., 2005). As such, milder heat processing to satisfy the consumers' demand for fresher products has become a new trend. However, reducing the intensity of thermal treatments raises concerns on the microbiological food safety. Processing times should be kept to a minimum to retain maximum quality of products, while ensuring consumer safety. Modelling the effect of heating conditions can be a useful tool to ensure safe food products. In this regard, the inactivation kinetics data of the pathogens under thermal treatment are necessary.

A majority of inactivation studies have evaluated the heat resistance of *Salmonella* and *L. monocytogenes* based on studies in buffered systems or broths (for example, Juneja et al., 2001; Miller et al., 2009; Sorqvist, 2003). However, an accurate prediction in/on solid food is not guaranteed based on the inactivation results in liquid systems. Murphy et al. (2000) have studied

the resistance of *Salmonella* and *Listeria* in ground chicken breast meat and observed an increased heat resistance in the solid compared to the liquid medium, at a temperature range of 55-70°C. Nevertheless, in several stages of food production, microbiological contamination is most likely to occur on the surface. Microorganisms on solid foods are immobilized rather than being present as planktonic cells in liquids. The differences between microbial growth or growth limits in liquids and on agar surface has been studied (Brocklehurst et al., 1997; Fujikawa and Morozumi, 2005; Koutsoumanis et al., 2004), and growth differences were observed. Information on the differences of heat resistance when comparing broth and (agar or food) surfaces has only limitedly been reported, with McCann et al. (2007) being one example. However, there is not a systematic study on the comparison of thermal inactivation of *S. enterica* and *L. monocytogenes* in liquid versus on solid surface.

Furthermore, it is well known that not all bacteria subjected to a thermal treatment, especially the mild thermal treatment, are killed, but a large number survive being physiologically injured (termed sublethal injury) (Besse, 2002). Sublethal injury typically affects cell wall or membrane permeability, and may also cause denaturation of various functional cell components (cellular proteins, enzymes, or nucleic acids). Sublethal injured cells are more sensitive to selective agents in media than healthy cells. In this way, sublethal cell injury is macroscopically detectable by the ability to form visible colonies on non-selective culture media but not on selective media (Jasson et al., 2007). The presence of sublethal injury cells present a potential threat to food safety, since in pathogen diagnostics they may result in underestimation of survivors or even false negative results due to the selective media used (Adams, 2005; Jasson et al., 2007). In respect of thermal inactivation modelling, if injured cells are not counted, the lethality of a process may be overestimated. Secondly, they may return to a more normal physiological state and be able to multiply, under suitable conditions, by repairing the damaged components after the thermal treatment (Ariefdjohan et al., 2004; Wu, 2008). Moreover, the stress may lead to adaptive changes

of the sublethally injured cells which may enhance their virulence or resistance to a wide variety of other stresses (Lianou and Koutsoumanis, 2013; Silva et al., 2015; Skandamis et al., 2008). Therefore, sublethal injury is an important aspect in managing food safety and should be considered when the effectiveness of food processing methods is examined (Silva et al., 2015). To the best of our knowledge, the sublethal injury of *S. enterica* and *L. monocytogenes* subjected to surface thermal treatment has not been described yet.

Therefore, the objective of the present study was to determine the thermal inactivation and sublethal injury kinetics of *S. enterica* and *L. monocytogenes* on solid (agar-seeded cells) media and to compare it with results obtained in liquid (suspended cells). The provided information will contribute to a better understanding of the sublethal injury of *S. enterica* and *L. monocytogenes* induced by thermal treatment on solid surface in comparison with results in liquid.

2. Material and methods

2.1. Bacterial strains and growth conditions

The three *S. enterica* (Gram negative) strains selected were all raw meat isolates: *Salmonella* Derby (LFMFP 872, pork isolate), *Salmonella* Enteritidis (LFMFP 875, poultry isolate) and *Salmonella* Typhimurium (LFMFP 877, poultry isolate). Three *L. monocytogenes* (Gram positive) strains that belong to the serotypes known to cause clinical cases, were used: LFMFP 392 (serotype 4b, liver pate isolate); LFMFP 421 (serotype 4b, clinical isolate) and LFMFP 491 (serotype 1/2b, tuna isolate). Frozen stocks of bacteria were maintained at -75°C in Tryptone Soy Broth (TSB, Oxoid, Basingstoke, England) with 0.6% yeast extract (YE, Oxoid) and 15% glycerol (Prolabo, Heverlee, Belgium). Working stocks were stored refrigerated at 4°C on Tryptone Soy Agar (TSA, Oxoid) slants and were renewed monthly. Each culture was activated individually by transferring a loopful from the slants into BHI (Brain Heart Infusion, Oxoid) and incubated at 37°C overnight. The

working cultures were prepared by transferring a loopful of each culture into 10 ml of BHI broth and incubated at 37°C for 24 h to yield late stationary phase cells.

2.2 Inoculation and thermal inactivation in broth

Thermal inactivation of *S. enterica* was studied at 55.0, 57.5 and 60.0°C, and *L. monocytogenes* was thermally treated at 60.0, 62.5 and 65.0°C. Before inoculation, a cocktail containing three strains of *S. enterica* or *L. monocytogenes* was prepared by mixing approximately equal populations of the three strains. The culture was then centrifuged at 3,600 g for 10 min (Sigma 4 K15 Centrifuge, Sigma) and washed with Peptone Physiological Salt Solution (PPS, containing 1 g/l neutralized bacteriological peptone and 8.5 g/l NaCl) once. The cell pellets of each pathogen were suspended in BHI and resulted in an initial concentration of approximately 10^9 CFU/ml. The thermal treatment experiments were carried out using thin walled PCR tubes (Bioplastics, Landgraaf, Netherlands) with 20 µL mixture culture and a thermal cycler instrument (Arktik Thermal Cycler; Thermo Fisher Scientific, Pittsburgh, PA, USA). The mode of temperature monitoring and control for the thermal cycler instrument is via the calculated sample temperature given a specific sample volume. To reduce and standardize the come-up time (time to reach the set process temperature), the heating program was initially set at 37°C for one min and then maintained at the target temperatures. Once the cultures reached the target temperature (after several seconds), duplicate tubes were removed at set time intervals and immersed immediately in an ice-water bath before they were plated. Samples taken at the start when the sample temperatures reached the target temperature were defined as time zero samples.

2.3 Inoculation and thermal inactivation on agar surface

Thermal resistance on solid surface was determined by using BHI agar (1.5%, w/v) plates. Plates were prepared by pouring approximately the same amount (16.0 ± 0.5 g) of molten agar into petri dishes (8.5 cm diameter), thus maintaining a standardized thickness of agar for all studies. 10 ml volume culture of each strain for a given pathogen were combined and centrifuged at 3,600 g for

10 min and washed once with PPS. The resulting cell pellet was then suspended in three ml of PPS to a final cell concentration of ca. 10^{10} CFU/ml. An aliquot of 0.1 ml of each culture suspension was spread on an agar plate to obtain an initial concentration of ca. 10^9 CFU/plate (ca. 1.8×10^7 CFU/cm^2). Inoculated plates were left at room temperature for 15 min to facilitate cells attachment to the agar surface. Each inoculated agar dish was aseptically removed from the petri dish using a sterile scalpel and put into a sterile stomacher bag (0.1 mm-thick, linear low-density polyethylene). The bags were vacuum sealed using a vacuum packaging system (Multivac A300/42, Vacuum compensation chamber, Hagenmüller KG, Germany). Thereafter, the inoculated samples were individually submerged in a water bath stabilized at the required temperatures (55.0, 57.5, 60.0, 62.5 or 65.0°C ± 0.1 °C). To ensure a constant temperature of the water bath during each experiment, bags were completely immersed in the water. The temperatures on the agar surface were continuously monitored by two thermocouples placed, prior to vacuum-sealing, between the bag and a non-inoculated (control) agar plate. The thermocouple readings were measured and recorded using a data logger (Testo 177-T4, Testo AG, Lenzkirch, Germany). The come-up times for the temperatures on the agar surfaces, in the range of 56 to 81 s at temperatures of 55.0 to 65.0°C, were excluded from the total treatment time to obtain consistent isothermal treatments. Samples for each replicate were removed at time intervals from 0.5 to 10 min and cooled in an ice water bath for 5 min before enumeration for survivors.

2.4 Cell recovery and estimation of sublethal injury

For the determination of surviving cells of thermal treated broth samples, the duplicate 20 µL samples were mixed and 25 µL was serially diluted in 225 µL PPS in microtiter plates. For the treatment on agar surface, sample bags were opened aseptically and 100 ml of PPS was added to each sample and then thoroughly homogenized in a stomacher for 2 min. Each sample was then serially 10-fold diluted with PPS. Appropriate dilutions were surface plated on appropriate media. Tryptic soy agar plus 0.6% (wt/vol) yeast extract (TSAYE) was used for the recovery of total viable

cells which includes the uninjured cells and the sublethally injured cells. In order to evaluate the occurrence of sublethal injury, samples were also plated onto selective recovery medium. Two widely used selective media for the detection of *Salmonella* or *L. monocytogenes* in food samples were applied: Xylose Lysine Deoxycholate (XLD, Oxoid) plates were used for *S. enterica* (Aljarallah and Adams, 2007; Jasson et al., 2011), and Agar *Listeria* Ottaviani & Agosti (ALOA, Biolife, Milano, Italy) plus selective supplement for *L. monocytogenes* (Jasson et al., 2007; Van Houtegheni et al., 2008). The colonies were counted after incubating plates at 37°C for 24 h for *S. enterica*, and 48 h for *L. monocytogenes*. The percentage of sublethally injured cells after thermal treatment was estimated by the following equation (Dykes, 1999), based on the log-transformed viable counts differences on non-selective ($N_{\text{non-selective}}$) and selective media ($N_{\text{selective}}$):

$$\%\ \text{Sublethal Injury} = \frac{N_{\text{non-selective}} - N_{\text{selective}}}{N_{\text{non-selective}}} \times 100 \qquad (1)$$

To quantify an average value of sublethal injury cells for each temperature, the Time-averaged injured cells coefficient (TICC) was calculated as follows (Miller et al., 2006):

$$\text{TICC} = \frac{\int_{t_{initial}}^{t_{final}} (\%\text{Sublethal Injury}\ (t))\mathrm{d}t}{t_{final} - t_{initial}} \qquad (2)$$

where t (min) is the thermal treatment time, the first and last sampling time are denoted by t_{inital} and t_{final}, respectively.

To evaluate the capability of the selective medium on recovering injured cells treated at different temperatures, the recovery inhibition coefficient (RIC), defined by Smith and Archer (1988), was introduced. It indicates the difference between the areas below inactivation curves obtained respectively in the selective and non-selective medium (Eq. 3). In general, this coefficient is a negative value, and the closer the RIC value approaches zero, the higher the capability of the selective medium in recovering injured cells.

$$\mathrm{RIC} = \int_0^{\text{total time}} \mathrm{Log}\left(N_{\text{selective}}(\mathrm{t})\right)\mathrm{dt} - \int_0^{\text{total time}} \mathrm{Log}\left(N_{\mathrm{TSAYE}}(\mathrm{t})\right)\mathrm{dt} \qquad (3)$$

2.5 Data analysis

Three biologically independent replicates were performed for inactivation studies for both pathogens. The counts per repetition were determined and converted to $\log_{10}$ values. A comparison was made of different potential primary models by the software GInaFiT (version 1.6) (Geeraerd et al., 2005, 2006) and the GraphPad Prism (GraphPad Software, Inc., San Diego, CA, USA). The goodness of fitting was assessed using adjusted regression coefficient (R^2_{adj}) and root mean squared error (*RMSE*). The adequacy of the models to describe the data was evaluated by using the lack-of-fit *F*-tests in which the mean square of the model and the measuring error are compared (Zwietering et al., 1994). For linear kinetics, linear regression was applied according to the equation:

$$\mathrm{Log}(N) = \mathrm{Log}(N_0) - k_{max}t/\mathrm{Ln}(10) \qquad (4)$$

where, N (CFU/ml or plate) is the number of survivors at time t (min) and N_0 (CFU/ml or plate) is the number of survivors at time 0, k_{max} (1/min) is the specific inactivation rate. A number of data sets showed a lag phase on inactivation curves prior to a log-linear region with a maximal inactivation rate. 'log-linear + shoulder' model (Geeraerd et al., 2000) (Eq. 4) was finally selected for fitting to observed data.

$$\mathrm{Log}(N) = \mathrm{Log}(N_0) - \frac{k_{max}t}{\mathrm{Ln}(10)} + \mathrm{Log}\left(\frac{e^{k_{max}S_l}}{1 + (e^{k_{max}S_l} - 1)\cdot e^{-k_{max}t}}\right) \qquad (5)$$

Herein, S_l (min) represents the shoulder length.

Considering the presence of shoulders on some of the inactivation curves, they were combined with the *D*-values (decimal reduction time) by calculating the time to obtain a 4*D* reduction (t_{4D}), Buchanan et al. (1993), as provided by GInaFiT. For comparative purposes (as will be shown below), an average *D*-value was then estimated as: $D_{average} = t_{4D}/4$, as in Metselaar et al. (2013) and Aryani et al. (2015). *z*-values (temperature increase necessary to reduce *D*-values by 10-fold) were

206 calculated as the negative reciprocal of the slope of the regression line between $\log_{10}$ *D*-values and
207 the treatment temperatures. To compare the heat resistance of *S. enterica* and *L. monocytogenes* in
208 broth in the present study with previous studies, a meta-analysis of *D*-values that collected form
209 literature where the experimental conditions were comparable to those in the present study (broth
210 or buffers of pH 7-7.5, a_w 0.99-1.00) was used. Besides *D*-values summarized by Doyle et al. (2000,
211 2001) in review papers, data from Lianou and Koutsoumanis. (2013), Aguirre et al. (2009), Aryani
212 et al. (2015), Casadei et al. (2001), Chiruta et al. (1997), Juneja et al. (2001), Miller et al. (2000),
213 Murphy et al. (1999, 2000), Stopforth et al. (2008), Xavier and Ingham (1997) were also included.
214 It should be noted that *D*-values of *Salmonella* Senftenberg 775W strain were not used in this study
215 due to its unusually high heat resistance (Ng et al., 1969).
216
217 The *z*-values were determined as the negative inverse slope of the plot of $\log_{10}$ *D*-values versus
218 temperature. Linear regressions were performed by GraphPad Prism using each replicate value as
219 an individual point. Statistical interpretation of differences among means of parameters, TICC and
220 RIC values was determined using ANOVA analysis (GraphPad Software) followed by a post hoc
221 (Tukey) test. In addition, the one-sample *t*-test was used to determine whether the shoulder length
222 differed significantly from zero. Tests were carried out using 95% confidence limits.
223

224 **3. Results and discussion**

225 **3.1 Thermal inactivation kinetics of *S. enterica***

226 Thermal inactivation of *S. enterica* in broth and on agar surface was measured at temperatures of
227 55.0, 57.5 and 60.0°C (Fig. 1A-F). For all the treatments, counts recovered on TSAYE media were
228 higher than those on XLD, and the difference generally increased with exposure time. Survival
229 curves of *S. enterica* recovered on XLD, which represents the uninjured subpopulation, were

clearly log-linear, while the inactivation curves recovered on TSAYE, which represent the total population, exhibited an obvious shoulder prior to log-linear decline (except in Fig. 1F, i.e., cells on agar surface at 60.0°C). The *t*-test also confirmed that the shoulder length is significant ($p < 0.05$). As can be seen in the figure, the linear model with a shoulder describes the inactivation kinetics very well. The *RMSE* values provided by GInaFit ranged from 0.07 to 0.39, the R^2_{adj} ranged from 0.955 to 0.998, which also indicated the satisfactory fittings. The lack-of-fit test *p*-values, ranged from 0.10 to 0.94, indicated that there was no significant lack of fit in the selected model. In the literature, shoulders were explained either by artefacts or by a normal feature of the mechanism of inactivation (Stringer et al., 2000). In this study, shoulders were only observed on inactivation curves obtained from nonselective media but not selective media, therefore it is unlikely that shoulders would be caused by an experimental artefact. Generally, the manifestation of shoulders to survival curves has been considered to indicate the accumulation of sublethal injury. According to Moats et al. (1971), a large number of critical sites need to be inactivated before the cells die. Initially the damage is incapable of killing the cell, but it becomes lethal when the damage accumulates. Some injured cells appear to be able to repair themselves and multiply on nonselective media but not on selective media. Thus, an initial energy barrier must be overcome before a lethal effect can be observed (Juneja et al., 2006). Furthermore, the fact that the shoulder length changes with temperature may also support this explanation: when inactivated in broth the shoulder length was reduced from 2.8 to 0.4 min as the temperature increased from 55.0 to 60.0°C (Table 1). Meanwhile, the relative shoulder length (compared to the average *D*-value) increased from 24.6% to 106.2%, which indicated that the shoulder was more relevant at higher temperatures. In addition, the genetic or phenotypical heterogeneity of the cells in a population is another possible cause for shoulders (Hornstra et al., 2009).

Average *D*-values were determined from counts recovered on both media (Table 1). It can be observed that, for a given temperature, *D*-values calculated from data of different media were of

256 the same magnitude. Nevertheless, all the *D*-values determined using TSAYE media were
257 significantly ($p < 0.05$) higher at all the three temperatures. Compared with the heat resistance in
258 broth, higher *D*-values were obtained on agar surface for each corresponding recovery media and
259 treatment temperature. McCann et al. (2007) have compared the thermal resistance of *S.*
260 typhimurium (DT104) in broth and on beef surfaces at 55°C, they also observed an increase in the
261 heat resistance of cells on solid surface. However, besides the physical forms difference (solid
262 versus liquid), the components and characteristics (e.g., pH, water activity) differences of the
263 matrix could also be attributed to the increased heat resistance. In the present study, the nutritional
264 components of the two matrixes are absolutely same, the only difference is with or without agar.
265 Therefore, the thermal resistance differences can be attributed to the different physical forms.

266

267 Compared with the published *D*-values at corresponding temperatures (Fig. 2A), *D*-values of
268 *Salmonella* obtained in broth were in broad agreement with the published data. The *D*-value at
269 55.0°C was higher than the average of reported *D*-values (5.6 ± 4.2 min, n = 37), while at 60.0°C
270 it was lower (1.1 ± 1.6 min, n = 47); but neither of the differences were significant ($p > 0.05$). It is
271 worth mentioning that the *D*-value of various strains showed a high variability, for instance, the *D*-
272 values at 60°C varied from 0.2 to 9.6 min. The inherent differences in the strains of the same species
273 have been recognized as a significant factor in heat resistance study. The intra-species variability
274 in heat resistance studies has been demonstrated for *S. enterica* (Lianou and Koutsoumanis, 2013)
275 and *L. monocytogenes* (Aryani et al., 2015). Due to this variation in thermal inactivation kinetics
276 among strains, cocktails of strains used for the thermal inactivation kinetics data determination will
277 increase confidence of the upper limit of heat resistance. In recent years multiple-strains of
278 microorganism cocktails have been preferred over single individual strains in determining the
279 thermal inactivation kinetics and lethality of pathogens in foods (Juneja et al., 2014; Juneja et al.,
280 2001; Murphy et al., 2000; Rajkowski, 2012). However, using multiple-strains to generate

281 inactivation kinetics data may lead to results that are difficult to interpret (Balasubramaniam et al.,
282 2004), and the potential impact of large strain variability may be overwhelming the factor studied
283 (den Besten et al., 2016).

284 Concerning the temperature sensitivity of *S. enterica*, evaluated by the *z*-values, results were not
285 significantly different ($p > 0.05$) between the uninjured and the total population for both thermal
286 treatment processes (Table 1), which means that the differences in surviving cells between samples
287 recovered on TSAYE and XLD remained approximately constant within the temperature range
288 studied. The *z*-value calculated in broth by using TSAYE was 3.4°C, which was similar to that
289 reported by Humpheson et al. (1998) where the *z*-value of *Salmonella* Enteritidis PT4 was 3.3°C.
290 However, the lower *z*-values calculated from this study, compared with the reported mean *z*-values
291 (6.4°C), were probably a result of differences in the strains used (Juneja, 2004).

292 **3.2 Thermally induced sublethal injury of *S. enterica***

293 The differences between the enumerated survivor populations are attributed to the sublethally
294 injured cells. Sublethal injuries, evaluated by using the selective-medium plating technique, reflect
295 the thermal injury effects on outer and/or cytoplasmic membranes (Baird-Parker et al., 2000). Heat-
296 induced injury of cells in broth and on agar surface is presented in Fig. 3A and B, respectively,
297 showing the injured proportion (%) of the total population after thermal inactivation at different
298 temperatures. Both in broth and on agar surface, the proportion of injured cells increased with
299 increasing treatment time, and the injury rate rose rapidly as the temperature increased from 55.0
300 to 60.0°C. The maximum proportions of sublethal injury were similar for treatment in broth at three
301 temperatures (ca. 99.5%) and there were significantly higher ($p < 0.05$) than those on agar surface
302 (ca. 96.2%). This is confirmed by the time-averaged injured cells coefficients (TICC values) for
303 the whole treatment (Table 2). *S. enterica* cells treated in broth showed significantly higher ($p <$
304 0.05) TICC values than on agar surface, at all the temperatures tested. Thus, for surface

pasteurization by a thermal treatment between 55 and 60°C, the occurrence of sublethal injury of *S. enterica* is a less important consideration compared with that in broth. It is interesting to note that differences among temperatures within each treatment were not significant. Arroyo et al. (2009) have reported similar results for *Cronobacter* spp. in TSBYE (Tryptone Soya Broth supplemented with 0.6 % of yeast extract) for treatment temperatures between 58 and 62°C. Thus, from a practical point of view, the results imply that the design of a process that using low or high temperatures would be similarly effective on the occurrence of sublethally injured *S. enterica* cells. As summarized in Table 3, the RIC values increased substantially as the temperature raised from 55.0 to 60.0°C both in broth and on surface, which means that higher treatment temperature results in higher capability of recovery on XLD plates. For cells heat-injured on agar surface, higher RIC values (i.e. higher recovery capability) were observed compared to those from broth of each corresponding temperature.

3.3 Thermal inactivation kinetics of *L. monocytogenes*

Fig. 4A-C show the inactivation curves of *L. monocytogenes* during exposure to 60.0, 62.5 and 65.0°C in BHI broth, respectively. Similar to *S. enterica*, more counts were recovered on nonselective (TSAYE) than selective media (ALOA), and the differences increased with heating time. Correspondingly, *D*-values based on the total population were higher than those derived from the uninjured subpopulation (Table 1), although it was only significant ($p < 0.05$) at 65.0°C. The log-linear model was successful at fitting data, which produced the R^2_{adj} greater than 0.984 and *RMSE* values less than 0.29, and the lack-of-fit *F*-test was accepted ($p > 0.05$). As illustrated in Fig. 2B, the obtained *D*-values of *L. monocytogenes*, for a given temperature, compared well to the reported *D*-values in broth or buffers. They were all very close to the average fitted *D*-values. The estimated *z*-values for *L. monocytogenes* heated in broth obtained on ALOA and TSAYE were 7.2 and 7.9°C, respectively, which were very close to the reported values of 7.6°C in deionized water

(Huang, 2004) and 7.5°C reported by Jemmi and Stephan (2006). On the other hand, our *z*-values were larger than reported *z*-values from other literature references, cited above (6.0°C) (Table 1).

In relation to the inactivation of *L. monocytogenes* on agar surface, inactivation curves are presented in Fig. 4D, E and F, the inactivation patterns were similar to those obtained from broth. Cells immobilized on agar surface exhibited higher heat resistance than planktonic cells in broth. The *D*-values of *L. monocytogenes* estimated from the total population at 60.0, 62.5 and 65.0°C (3.1, 1.2 and 0.4 min) differed significantly ($p < 0.05$) from those in broth (1.3, 0.5 and 0.3 min) (Table 1). This was similar for the *D*-values derived from the cells recovered on ALOA plates, they were significantly increased by 1.8 to 2.3 fold. To put the findings in perspective, we benchmarked our results with the meta-analysis data. As seen in Fig. 2, all the values obtained from broth and model agar surfaces lie within the 95% prediction band. The heat resistance differences between the broth and model agar became, although significant, less apparent. *F*-test, introduced by Aryani et al. (2015), was used to compare the effect of heating matrix (broth or agar) on heat resistance to the strain variability found in literature. For results at 60.0 and 65.0°C the *F*-test values (0.42 and 0.10, respectively) indicated that effect of heating matrix on heat resistance at the two temperatures was generally similar to the strain variability. This is consistent with a recent study, which revealed the impact of strain variability in heat resistance was very large and overwhelming many other effects (den Besten et al., 2016). They suggested that when studying one variability factor on growth or heat resistance, it was useful to benchmark the factor to the overall variability found in literature.

3.4 Thermally induced sublethal injury of *L. monocytogenes*

The degree of thermal injury of *L. monocytogenes* during thermal treatment is presented in Fig. 5. The proportion of sublethal injury increased as the heating time went on. McKellar et al. (1997)

also indicated that longer exposure to the treatment temperature (58°C) resulted in a significantly increased level of *L. monocytogenes* cells injury. Regarding the TICC values for the whole treatment, significant increase of damage was observed when the temperature increased from 60.0 to 65.0°C (from 61.8% to 79.8% in broth, 86.5% to 97.1 % on agar surface, Table 2). A number of authors have showed similar results: increasing treatment temperatures resulted in an increase in the proportion of sublethally damaged cells. A study by Miller et al. (2006) showed similar results: increasing treatment temperatures resulted in an increase in the proportion of sublethally damaged cells. They investigated the recovery of injured *L. innocua* cells thermally treated in broth, and the TICC values based on the Palcam plates increased from 83.8 at 52.5°C to 99.0 at 65.0°C. In contrast with *S. enterica*, TICC values of *L. monocytogenes* cells in broth were significantly lower ($p < 0.05$) than those on solid surface (Table 2). *L. monocytogenes* cells thermally treated on agar surface were more heat resistant and more easily sublethally injured than those in broth. Consequently, the use of inactivation results obtained in broth to assess the efficacy of inactivation on solid surface, in terms of the underestimation of *D*-values and sublethal injury, may lead to an overestimation of the effect of a thermal process.

With regards to the RIC values, when the temperature increases, similar temperature effects as in the case of *S. enterica* can be observed (Table 3). However, better recovery was observed in broth than on agar surface, which is contrast with *S. enterica*. Miller et al. (2010) studied the recovery of heat-injured *L. innocua* cells on parsley, the RIC values derived from Palcam plates at 60.0 and 65.0 were -19.3 and -3.8, respectively, which were a little lower than those on agar surface in this study.

When treated at the same temperature (60.0°C), TICC value of *S. enterica* in broth was much higher than that of *L. monocytogenes*. In this sense, the sublethal injury of *L. monocytogenes* cells induced by heating was significantly lower than that of *S. enterica*. However, in the present study, due to

different selective media used for *S. enterica* and *L. monocytogenes*, the different effective stresses on the cells could be one of the factors contributing to the differences in the average level of sublethal injury. In a previous study by Jasson et al. (2007), *L. monocytogenes* also exhibit much less sublethal injury than other Gram-negative bacteria such as *E. coli* O157:H7 and *Campylobacter jejuni*. Noriega et al. (2013) also indicated that the sublethal damage of *Listeria innocua* colonies in a gellified food model system exposed to mild thermal treatment (54.0°C) were significantly lower than *E. coli* and *Salmonella* Typhimurium.

4. Conclusions

In conclusion, the present study compares the inactivation and sublethal injury kinetics of *S. enterica* and *L. monocytogenes* thermally treated in broth and on model agar surface. To our knowledge, this is the first report using agar surface, mimic a food surface, to investigate the inactivation and sublethal injury kinetics. In general, the cells treated on agar surface exhibited higher heat resistance than cells in broth. It has been demonstrated that thermal inactivation in broth or on solid surface affects the degree of sublethal injury. Additionally, the degree of sublethal injury depends on the target microorganism under investigation. As a consequence, from a practical point of view, when safe thermal treatments or hurdle strategies are designed to reduce the risk of *S. enterica* and *L. monocytogenes*, care has to be taken in terms of a case-specific (food product/micro-organism couple) determination of heat inactivation parameters, including the occurrence and the extent of sublethal injury. It is also confirmed that the need to validate the existing broth-based models or parameters for pathogens survival to be applied to processing situation in industry. Moreover, meta-analysis on parameters could be used as a benchmark to evaluate the data in perspective.

Acknowledgement

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References

Adams, M., 2005. Detecting sublethally damaged cells. Understanding Pathogen Behaviour. Woodhead Publishing Limited, Cambridge, England, pp. 199-212.

Aguirre, J., Pin, C., Rodríguez, M., de Fernando, G.G., 2009. Analysis of the variability in the number of viable bacteria after mild heat treatment of food. Appl. Environ. Microbiol. 75, 6992-6997.

Aljarallah, K.M., Adams, M.R., 2007. Mechanisms of heat inactivation in *Salmonella* serotype Typhimurium as affected by low water activity at different temperatures. J. Appl. Microbiol. 102, 153-160.

Álvarez-Ordóñez, A., Fernández, A., López, M., Arenas, R., Bernardo, A., 2008. Modifications in membrane fatty acid composition of *Salmonella* typhimurium in response to growth conditions and their effect on heat resistance. Int. J. Food Microbiol. 123, 212-219.

Ariefdjohan, M.W., Nelson, P.E., Singh, R.K., Bhunia, A.K., Balasubramaniam, V.M., Singh, N., 2004. Efficacy of high hydrostatic pressure treatment in reducing *Escherichia coli* O157 and *Listeria monocytogenes* in alfalfa seeds. J. Food Sci. 69, M117-M120.

Arroyo, C., Condon, S., Pagan, R., 2009. Thermobacteriological characterization of *Enterobacter sakazakii*. Int. J. Food Microbiol. 136, 110-118

Aryani, D.C., den Besten, H.M.W., Hazeleger, W.C., Zwietering, M.H., 2015. Quantifying variability on thermal resistance of *Listeria monocytogenes*. Int. J. Food Microbiol. 193, 130-138.

Baird-Parker, T.C., Gould, G.W., Lund, B.M., 2000. The microbiological safety and quality of food. Aspen Publishers, Inc, Gaithersburg, Maryland.

Balasubramaniam, V., Ting, E., Stewart, C., Robbins, J., 2004. Recommended laboratory practices for conducting high-pressure microbial inactivation experiments. Innov. Food Sci. Emerg. Technol. 5, 299-306.

Bermúdez-Aguirre, D., Corradini, M.G., 2012. Inactivation kinetics of *Salmonella* spp. under thermal and emerging treatments: A review. Food Res. Int. 45, 700-712.

Besse, N.G., 2002. Influence of various environmental parameters and of detection procedures on the recovery of stressed *L. monocytogenes*: A review. Food Microbiol. 19, 221-234.

Brocklehurst, T., Mitchell, G., Smith, A., 1997. A model experimental gel-surface for the growth of bacteria on foods. Food Microbiol. 14, 303-311.

443 Buchanan, R.L., Golden, M.H., Whiting, R.C., 1993. Differentiation of the effects of pH and lactic
444 or acetic-acid concentration on the kinetics of *Listeria monocytogenes* inactivation. J. Food
445 Prot. 56, 474-478.

446 Casadei, M.A., Ingram, R., Hitchings, E., Archer, J., Gaze, J.E., 2001. Heat resistance of *Bacillus*
447 *cereus*, *Salmonella* typhimurium and *Lactobacillus delbrueckii* in relation to pH and
448 ethanol. Int. J. Food Microbiol. 63, 125-134.

449 Chiruta, J., Davey, K.R., Thomas, C.J., 1997. Thermal inactivation kinetics of three vegetative
450 bacteria as influenced by combined temperature and pH in a liquid medium. Food Bioprod.
451 Process. 75, 174-180.

452 den Besten, H.M.W., Aryani, D.C., Metselaar, K.I., Zwietering, M.H., Microbial variability in
453 growth and heat resistance of a pathogen and a spoiler: All variabilities are equal but some
454 are more equal than others. Int. J. Food Microbiol. doi:10.1016/j.ijfoodmicro.2016.04.025.

455 Devlieghere, F., Vermeiren, L., Debevere, J., 2004. New preservation technologies: Possibilities
456 and limitations. Int. Dairy J. 14, 273-285.

457 Doyle, M.E., Mazzotta, A.S., 2000. Review of studies on the thermal resistance of *Salmonellae*. J.
458 Food Prot. 63, 779-795.

459 Doyle, M.E., Mazzotta, A.S., Wang, T., Wiseman, D.W., Scott, V.N., 2001. Heat resistance of
460 *Listeria monocytogenes*. J. Food Prot. 64, 410-429.

461 Dykes, G.A., 1999. Physical and metabolic causes of sub-lethal damage in *Listeria monocytogenes*
462 after long-term chilled storage at 4°C. Int. J. Food Microbiol. 87, 915-922.

463 EFSA (European Food Safety Authority) and ECDC (European Centre for Disease Prevention and
464 Control), 2015. The European Union summary report on trends and sources of zoonoses,
465 zoonotic agents and food-borne outbreaks in 2014. EFSA J. 2015;13(12):4329.

466 Fujikawa, H., Morozumi, S., 2005. Modeling surface growth of *Escherichia coli* on agar plates.
467 Appl. Environ. Microbiol. 71, 7920-7926.

468 Geeraerd, A.H., Herremans, C.H., Van Impe, J.F., 2000. Structural model requirements to describe
469 microbial inactivation during a mild heat treatment. Int. J. Food Microbiol. 59, 185-209.

470 Geeraerd, A.H., Valdramidis, V.P., Van Impe, J.F., 2005. GInaFiT, a freeware tool to assess non-
471 log-linear microbial survivor curves. Int. J. Food Microbiol. 102, 95-105.

472 Geeraerd, A.H., Valdramidis, V.P., Van Impe, J.F., 2006. Erratum to "GInaFiT, a freeware tool to
473 assess non-log-linear microbial survivor curves"[Int. J. Food Microbiol. 102 (2005) 95–
474 105]. Int. J. Food Microbiol. 110, 297.

475 Harris, L.J., Farber, J.N., Beuchat, L.R., Parish, M.E., Suslow, T.V., Garrett, E.H., Busta, F.F.,
476 2003. Outbreaks associated with fresh produce: Incidence, growth, and survival of
477 pathogens in fresh and fresh-cut produce. Compr Rev Food Sci Food Saf 2, 78-141.

478 Hornstra, L.M., Ter Beek, A., Smelt, J.P., Kallemeijn, W.W., Brul, S., 2009. On the origin of
479 heterogeneity in (preservation) resistance of *Bacillus* spores: input for a 'systems' analysis
480 approach of bacterial spore outgrowth. Int. J. Food Microbiol. 134, 9-15.

481 Huang, L., 2004. Thermal resistance of *Listeria monocytogenes*, *Salmonella* Heidelberg, and
482 *Escherichia coli* O157:H7 at elevated temperatures. J. Food Prot. 67, 1666-1670.

483 Humpeson, L., Adams, M.R., Anderson, W.A., Cole, M.B., 1998. Biphasic thermal inactivation
484 kinetics in *Salmonella* enteritidis PT4. Appl. Environ. Microbiol. 64, 459-464.

Jasson, V., Baert, L., Uyttendaele, M., 2011. Detection of low numbers of healthy and sub-lethally injured *Salmonella enterica* in chocolate. Int. J. Food Microbiol. 145, 488-491.

Jasson, V., Uyttendaele, M., Rajkovic, A., Debevere, J., 2007. Establishment of procedures provoking sub-lethal injury of *Listeria monocytogenes*, *Campylobacter jejuni* and *Escherichia coli* O157 to serve method performance testing. Int. J. Food Microbiol. 118, 241-249.

Jemmi, T., Stephan, R., 2006. *Listeria monocytogenes*: food-borne pathogen and hygiene indicator. Rev. - Off. Int. Epizoot. 25, 571-580.

Juneja, V.K., 2004. Extrinsic and intrinsic factors that influence microbial heat resistance. Bulletin of the International Dairy Federation No 392/2004, 16-30.

Juneja, V.K., Eblen, B., Ransom, G., 2001. Thermal inactivation of *Salmonella* spp. in chicken broth, beef, pork, turkey, and chicken: Determination of D-and Z-values. J. Food Sci. 66, 146-152.

Juneja, V.K., Huang, L., Marks, H., 2006. Approaches for modeling thermal inactivation of food borne pathogens, ACS symposium series. Oxford University Press, pp. 235-251.

Juneja, V. K., Mukhopadhyay, S., Marks, H., Mohr, T.B., Warning, A., Datta, A., 2014. Predictive thermal inactivation model for effects and interactions of temperature, NaCl, sodium pyrophosphate, and sodium lactate on *Listeria monocytogenes* in ground beef. Food Bioproc Tech 7, 437-446.

Koutsomanis, K.P., Kendall, P.A., Sofos, J.N., 2004. A comparative study on growth limits of *Listeria monocytogenes* as affected by temperature, pH and a_w when grown in suspension or on a solid surface. Food Microbiol. 21, 415-422.

Lianou, A., Koutsoumanis, K.P., 2013. Evaluation of the strain variability of *Salmonella enterica* acid and heat resistance. Food Microbiol. 34, 259-267.

McCann, M.S., McGovern, A.C., McDowell, D.A., Blair, I.S., Sheridan, J.J., 2007. Effects of storage and the presence of a beef microflora on the thermal resistance of *Salmonella* Typhimurium DT104 in beef and broth systems. Int. J. Food Microbiol. 102, 1561-1569.

McKellar, R., Butler, G., Stanich, K., 1997. Modelling the influence of temperature on the recovery of *Listeria monocytogenes* from heat injury. Food Microbiol. 14, 617-626.

Metselaar, K.I., den Besten, H.M., Abee, T., Moezelaar, R., Zwietering, M.H., 2013. Isolation and quantification of highly acid resistant variants of *Listeria monocytogenes*. Int. J. Food Microbiol. 166, 508-514.

Miller, A.J., Bayles, D.O., Eblen, B.S., 2000. Cold shock induction of thermal sensitivity in *Listeria monocytogenes*. Appl. Environ. Microbiol. 66, 4345-4350.

Miller,F.A., Brandao, T.R.S., Teixeira, P., Silva, C.L.M., 2006. Recovery of heat-injured *Listeria innocua*. Int. J. Food Microbiol. 112, 261-265.

Miller,F.A., Gil, M.M., Brandao, T.R.S., Teixeira, P., Silva, C.L.M., 2009. Sigmoidal thermal inactivation kinetics of *Listeria innocua* in broth: Influence of strain and growth phase. Food Control 20, 1151-1157.

Miller, F.A., Ramos, B., Brandao, T.R.S., Teixeira, P., Silva, C.L.M., 2010. Comparison of recovery methods for the enumeration of injured *Listeria innocua* cells under isothermal and non-isothermal treatments. Food Microbiol. 27, 1112-1120.

527 Moats, W.A., Dabbah, R., Edwards, V.M., 1971. Interpretation of nonlogarithmic survivor curves
528 of heated bacteria. J. Food Sci. 36, 523-526.

529 Murphy, R.Y., Marks, B.P., Johnson, E.R., Johnson, M.G., 1999. Inactivation of *Salmonella* and
530 *Listeria* in ground chicken breast meat during thermal processing. J. Food Prot. 62, 980-
531 985.

532 Murphy, R.Y., Marks, B.P., Johnson, E.R., Johnson, M.G., 2000. Thermal inactivation kinetics of
533 *Salmonella* and *Listeria* in ground chicken breast meat and liquid medium. J. Food Sci. 65,
534 706-710.

535 Ng, H., Bayne, H.G., Garibaldi, J.A., 1969. Heat resistance of *Salmonella*: The uniqueness of
536 *Salmonella* senftenberg 775W. Appl Microbiol. 17, 78-82.

537 Noriega, E., Velliou, E., Van Derlinden, E., Mertens, L., Van Impe, J.F.M., 2013. Effect of cell
538 immobilization on heat-induced sublethal injury of *Escherichia coli*, *Salmonella*
539 Typhimurium and *Listeria innocua*. Food Microbiol. 36, 355-364.

540 Rajkowski, K.T., 2012. Thermal inactivation of *Escherichia coli* O157:H7 and *Salmonella* on
541 catfish and tilapia. Food Microbiol. 30, 427-431.

542 Rawson, A., Patras, A., Tiwari, B.K., Noci, F., Koutchma, T., Brunton, N., 2011. Effect of thermal
543 and non thermal processing technologies on the bioactive content of exotic fruits and their
544 products: Review of recent advances. Food Res. Int. 44, 1875-1887.

545 Rose, B.E., Hill, W.E., Umholtz, R., Ransom, G.M., James, W.O., 2002. Testing for *Salmonella* in
546 raw meat and poultry products collected at federally inspected establishments in the United
547 States, 1998 through 2000. J. Food Prot. 65, 937-947.

548 Silva, A., Genovés, S., Martorell, P., Zanini, S.F., Rodrigo, D., Martinez, A., 2015. Sublethal injury
549 and virulence changes in *Listeria monocytogenes* and *Listeria innocua* treated with
550 antimicrobials carvacrol and citral. Food Microbiol. 50, 5-11.

551 Smith, J.L., Archer, D.L., 1988. Heat-induced injury in *Listeria monocytogenes*. J. Ind. Microbiol.
552 3, 105-110.

553 Skandamis, P.N., Yoon, Y., Stopforth, J.D., Kendall, P.A., Sofos, J.N., 2008. Heat and acid
554 tolerance of *Listeria monocytogenes* after exposure to single and multiple sublethal stresses.
555 Food Microbiol. 25, 294-303.

556 Sorqvist, S., 2003. Heat resistance in liquids of *Enterococcus* spp., *Listeria* spp., *Escherichia coli*,
557 *Yersinia enterocolitica*, *Salmonella* spp. and *Campylobacter* spp. Acta Vet. Scand. 44, 1-
558 19.

559 Stopforth, J.D., Suhalim, R., Kottapalli, B., Hill, W.E., Samadpour, M., 2008. Thermal inactivation
560 D- and z-values of multidrug-resistant and non-multidrug-resistant *Salmonella* serotypes
561 and survival in ground beef exposed to consumer-style cooking. J. Food Prot. 71, 509-515.

562 Stringer, S.C., George, S.M., Peck, M.W., 2000. Thermal inactivation of *Escherichia coli* O157:H7.
563 Symp Ser Soc Appl Microbiol. 88, 79S-89S.

564 Van Houtegheni, N., Devlieghere, F., Rajkovic, A., Gomez, S.M.O., Uyttendaele, M., Debevere,
565 J., 2008. Effects of CO_2 on the resuscitation of *Listeria monocytogenes* injured by various
566 bactericidal treatments. Int. J. Food Microbiol. 123, 67-73.

567 Walsh, C., Duffy, G., Sheridan, J., Fanning, S., Blair, I., McDowell, D., 2005. Thermal resistance
568 of antibiotic-resistant and antibiotic-sensitive *Salmonella* spp. on chicken meat. J. Food
569 Saf. 25, 288-302.

570 Wu, V.C.H., 2008. A review of microbial injury and recovery methods in food. Food Microbiol.
571 25, 735-744.

572 Xavier, I.J., Ingham, S.C., 1997. Increased D-values for *Salmonella enteritidis* following heat
573 shock. J. Food Prot. 60, 181-184.

574 Zwietering, M., Cuppers, H., De Wit, J., Van't Riet, K., 1994. Evaluation of data transformations
575 and validation of a model for the effect of temperature on bacterial growth. Appl. Environ.
576 Microbiol. 60, 195-20

Tables

Table 1 Thermal inactivation parameters of *S. enterica* (SALM) and *L. monocytogenes* (LM) in broth and on agar surface.

Pathogens	Heating strategies	Population	Shoulder length (min) [1]			Average *D*-value (min)[1]			*z*-value ± SE (°C) [3]	*z*-value ± SE from literature (°C) [3]
			55.0°C [2]	57.5°C	60.0°C	55.0°C [2]	57.5°C	60.0°C		
		Uninjured	NA[4]	NA	NA	8.7 ± 0.3 [a]	2.0 ± 0.1 [a]	0.3 ± 0.0 [a]	3.2 ± 0.1 [a]	
		Total	2.8 ± 1.5	1.8 ± 0.7	0.4 ± 0.1	11.4 ± 0.2 [c]	2.9 ± 0.1 [cd]	0.4 ± 0.0 [bc]	3.4 ± 0.1 [ab]	6.4 ± 0.2
		Uninjured	NA	NA	NA	10.1 ± 0.4 [b]	2.4 ± 0.1 [b]	0.4 ± 0.0 [c]	3.7 ± 0.1 [ab]	
		Total	4.7 ± 1.3	2.1 ± 1.8	NA	11.6 ± 0.2 [c]	3.2 ± 0.1 [d]	0.6 ± 0.0 [d]	3.8 ± 0.1 [b]	
			60.0°C	62.5°C	65.0°C	60.0°C	62.5°C	65.0°C		
		Uninjured	NA	NA	NA	1.2 ± 0.0 [e]	0.5 ± 0.0 [e]	0.2 ± 0.0 [e]	7.2 ± 0.3 [f]	
		Total	NA	NA	NA	1.3 ± 0.0 [e]	0.5 ± 0.0 [e]	0.3 ± 0.0 [f]	7.9 ± 0.3 [f]	6.0 ± 0.2
		Uninjured	NA	NA	NA	2.7 ± 0.1 [f]	0.9 ± 0.1 [f]	0.4 ± 0.1 [fg]	5.7 ± 0.3 [e]	
		Total	NA	NA	NA	3.1 ± 0.3 [g]	1.2 ± 0.1 [g]	0.4 ± 0.0 [g]	5.8 ± 0.2 [e]	

[1] Values are expressed in means ± standard deviations from three replicates.
[2] Means in the same column with a different letter (a through d for *S. enterica* and e through g for *L. monocytogenes*) are significantly different ($p < 0.05$).
[3] *z*-values are mean ± standard error.
[4] Not applicable.

Table 2 Time-averaged injured cells coefficient (TICC) for thermal treatment of *S. enterica* and *L. monocytogenes* in broth and on agar surface.

Temp (°C)	TICC (%) of heat-injured *S. enterica* [1]		Temp (°C)	TICC (%)of heat-injured *L. monocytogenes* [1]	
	In broth [2]	On surface [2]		In broth [2]	On surface [2]
55.0	94.7 ± 0.1 ^{aB}	90.1 ± 0.2 ^{aA}	60.0	61.8 ± 4.1 ^{dD}	86.5 ± 1.6 ^{dE}
57.5	94.8 ± 0.7 ^{aB}	90.6 ± 1.6 ^{aA}	62.5	72.1 ± 1.8 ^{eD}	93.0 ± 2.0 ^{eE}
60.0	93.6 ± 0.5 ^{aB}	90.7 ± 1.2 ^{aA}	65.0	79.8 ± 2.7 ^{eD}	97.1 ± 0.7 ^{fE}

[1] Values are expressed in means ± standard deviations from three replicates.
[2] Means in the same column with a different letter (a through c for *S. enterica* and d through f for *L. monocytogenes*) are significantly different ($p < 0.05$). Means in the same row with different letters (A through B for *Salmonella* and D through E for *L. monocytogenes*) are significantly different ($p < 0.05$).

Table 3 Evaluation of the ability of the media to recover injured cells (RIC) of thermal treatment *S. enterica* and *L. monocytogenes* cells in broth and on agar surface.

Temp (°C)	RIC of heat-injured *S.enterica* [1]		Temp (°C)	RIC of heat-injured *L. monocytogenes* [1]	
	In broth [2]	On surface [2]		In broth [2]	On surface [2]
55.0	-88.0 ± 2.2 ^{cA}	-56.9 ± 2.2 ^{cB}	60.0	-4.4 ± 0.4 ^{eE}	-13.5 ± 0.7 ^{fD}
57.5	-21.6 ± 0.7 ^{bA}	-11.4 ± 0.9 ^{bB}	62.5	-1.9 ± 0.2 ^{dE}	-6.6 ± 0.6 ^{eD}
60.0	-3.0 ± 0.2 ^{aA}	-2.4 ± 0.3 ^{aB}	65.0	-1.3 ± 0.0 ^{dE}	-3.0 ± 0.4 ^{dD}

[1] Values are expressed in means ± standard deviations from three replicates.
[2] Means in the same column with a different letter (a through c for *S. enterica* and d through f for *L. monocytogenes*) are significantly different ($p < 0.05$). Means in the same row with different letters (A through B for *Salmonella* and D through E for *L. monocytogenes*) are significantly different ($p < 0.05$).

603 **Figure legends**

604 **Figure 1.** Thermal inactivation curves of *S. enterica* in broth (A, B and C) and on agar surface (D,
605 E and F) at temperatures of 55.0 (A, D), 57.5 (B, E) and 60.0°C (C, F), using TSAYE (○) and XLD
606 (□) as recovery media. Data points of each time represents three independent replicates, solid lines
607 are regression lines fitted with models.

608

609 **Figure 2.** Summary of heat resistance data for *Salmonella* spp. (A) and *L. monocytogenes* (B) in
610 broth and buffer from literature (○) and this study (□). The black solid curves represent the fitting
611 line; the red dotted curves are upper and lower confidence limits at a 95% level; the green lines
612 represent the 95% upper and lower prediction intervals.

613

614 **Figure 3.** Evolution of sublethal injury (%) of *S. enterica* thermally treated in broth (A) and on
615 agar surface (B) at different temperatures. Each data point represents the mean of three independent
616 replicates, and the error bars denote SD.

617 **Figure 4.** Thermal inactivation curves of *L. monocytogenes* in broth (A, B and C) and on agar
618 surface (D, E and F) at temperatures of 60.0 (A, D), 62.5 (B, E) and 65.0°C (C, F), using TSAYE
619 (○) and ALOA (□) as recovery media. Solid lines are regression lines fitted with models.

620

621 **Figure 5.** Evolution of sublethal injury (%) of *L. monocytogenes* thermally treated in broth (A) and
622 on agar surface (B) at different temperatures. Each data point represents the mean of three
623 independent replicates, and the error bars denote SD.

Figure 1.

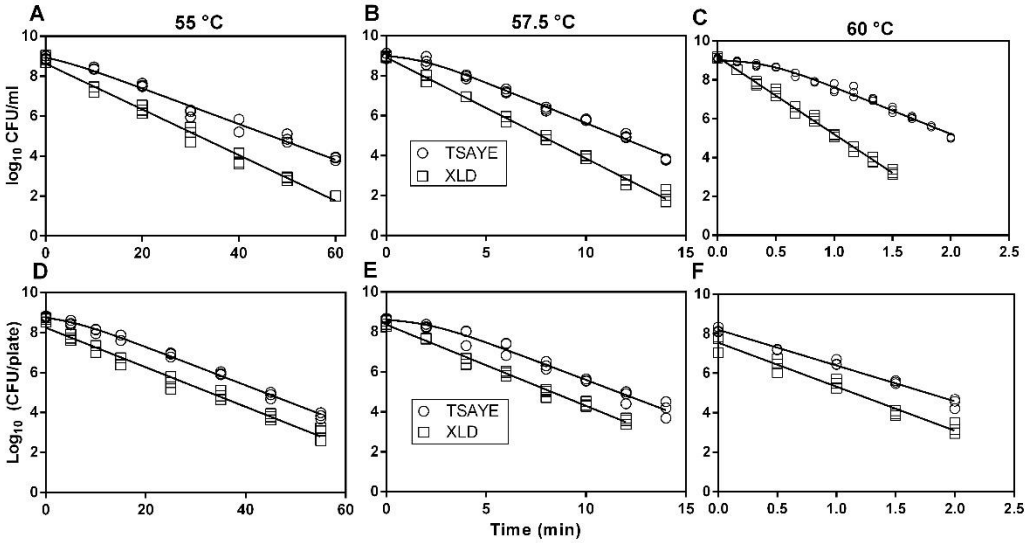


Figure 2.

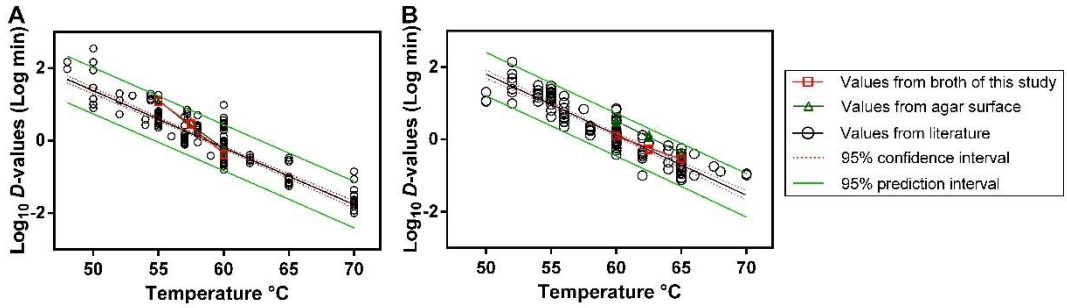
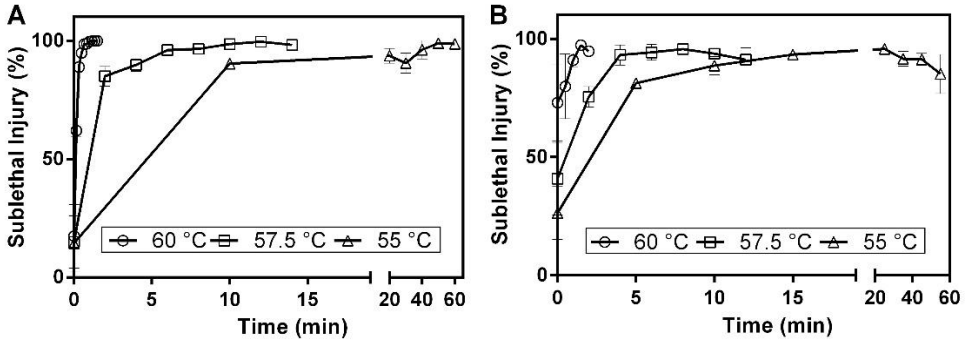
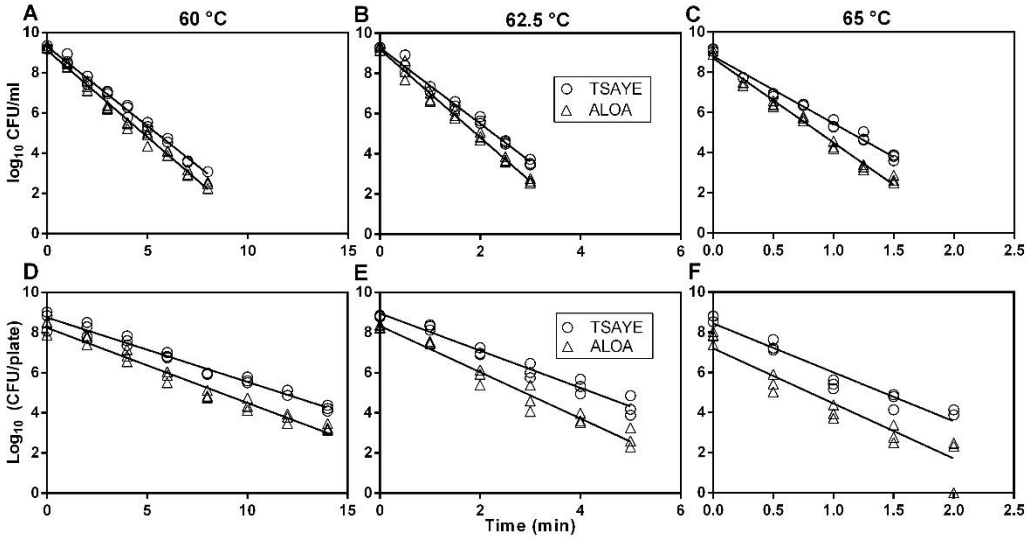


Figure 3.

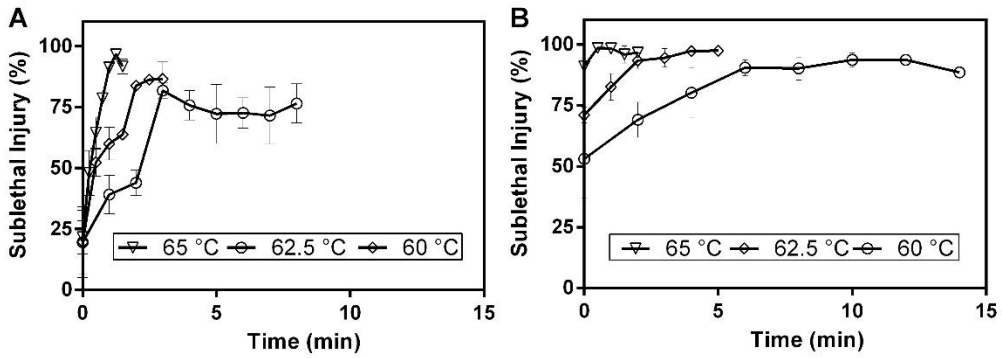


631 **Figure 4.**



632

633 **Figure 5.**



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